Immunological Changes After Both Exercise and Activity in Chronic Fatigue Syndrome: A Pilot Study

P. D. White, MD
K. E. Nye, PhD
A. J. Pinching, DM
T. M. Yap, MSc
N. Power, MRCPsych
V. Vleck, PhD
D. J. Bentley, PhD
J. M. Thomas, MSc
M. Buckland, MRCP
J. M. Parkin, MRCP

ABSTRACT. Background: The chronic fatigue syndrome (CFS) is characterized by post-exertional malaise and fatigue. We designed this
pilot study to explore whether the illness was associated with alterations in immunological markers following exercise.

Methods: We measured immunological markers before and up to three days after either a sub-maximal or maximal bicycle exercise test. We studied nine patients with CFS and nine age- and sex-matched healthy but sedentary controls. We also studied the same patients with CFS at home after a night’s sleep and then after traveling to the study center.

Results: There were no significant differences in any of the cell markers after a sub-maximal exercise test compared to a maximal test. However, we found elevated concentrations of plasma transforming growth factor beta (TGF-β), even before exercise, in subjects with CFS (median (IQR) of 904 (182-1072) pg/ml versus controls (median (IQR) of 50 (45-68) pg/ml) \((P < .001)\). Traveling from home to the hospital significantly elevated TGF-β concentrations from a resting median (IQR) concentration of 1161 (130-1246) pg/ml to a median (IQR) concentration of 1364 (1155-1768) pg/ml \((P < .02)\). There was also a sustained increase in plasma tumor necrosis factor alpha (TNF-α) after exercise in CFS patients, but not in controls \((P = .004\) for the area under the curve), although traveling had no such effect. CD3, CD4 and HLA DR-expressing lymphocyte counts were lower in CFS patients, but exercise had the same effect in both groups, causing an immediate increase in circulating cell numbers that lasted less than three hours.

Conclusions: These results suggest that the relationship between physical activity and both pro-inflammatory and anti-inflammatory cytokines merits further investigation in patients with CFS. The results also emphasize the importance of defining a truly resting baseline condition in such studies.

KEYWORDS. Chronic fatigue syndrome, exercise, cytokines, TNF-α, TGF-β

INTRODUCTION

The chronic fatigue syndrome (CFS) is a condition of chronic and disabling fatigue, which is not caused by recognized medical conditions or major psychiatric disorder (1-3). A common complaint of sufferers is post-exertional fatigue or malaise (1-3). The illness can be precipitated by certain infections, but we do not know why only a small proportion of those infected are left with CFS (1).
Immunological abnormalities are commonly observed in CFS; these could either be a sequela of an infective or other trigger, or be secondary to changes in sleep and activity (1, 4). Findings include activation of CD8 cells, altered proliferation and cytokine secretion, and reduced NK cell function (5). However, the role of these changes in the etiology of CFS remains uncertain. The similarity between symptoms of CFS and the effects of some cytokines, along with the commonly observed onset after infections, has led to the hypothesis that abnormal cytokine regulation may be important in etiology (4).

Changes in circulating levels of several different cytokines have been reported in CFS. Concentrations of plasma transforming growth factor-beta (TGF-β) (anti-inflammatory) and tumor necrosis factor-alpha (TNF-α) (pro-inflammatory) have both been shown to be raised (6-9), but not in all studies (10, 11). These changes in cytokine concentrations may be linked to the lymphocyte activation sometimes observed in CFS (5, 11). Abnormal regulation of cytokines may both reflect and cause altered function and interaction across a broad range of cell types. One possible mechanism is suggested by the observation of central motor fatigue caused by intra-cerebral TGF-β in mice (12).

Five studies have examined the effect of exercise on immune measures in CFS. Ten mildly disabled patients with CFS had significantly elevated serum TGF-β compared to 10 healthy controls before exercise testing, with a further rise 40 minutes after gentle exercise, which was not significantly different from controls (9). No differences in gamma interferon or lymphocyte sub-sets (CD3+, CD8+, NK cells) were found up to 24 hours after exercise in 20 women with CFS, compared to 14 healthy sedentary women (13). Thirty minutes of hand-grip exercise induced no significant differences in interferon gamma and alpha, interleukin 1β, and TNF-α in 12 male patients with CFS (14). There was no difference in IL-6 and IL1-β in CFS after 15 minutes stepping exercise compared to controls (15). Sorensen and colleagues have recently reported that bicycle sub-maximal exercise induced a significant elevation of complement C4a concentration six hours later, but there was no elevation of C3a or C5a (16). These modest findings may be related to the nature and intensity of the exercise employed. No studies have compared the effects of exercise of different intensity or duration on the immune response in subjects with CFS.

Altered cytokine levels, whatever their origin, could modify muscle and/or neural function. This could alter the response of the patient to physical activity. On the other hand, muscle activity and/or injury may
modify cytokine levels. An interaction between some cytokines and muscle injury has been shown in the setting of endurance exercise (17).

CFS patients are intolerant of exercise (18). This may reflect an undisclosed pathology, possibly related to cytokine imbalance, and/or be related to deconditioning (18, 19). Altered cytokine release may result from deconditioning. An increased concentration of the cytokine interleukin 1 was found five hours after unaccustomed exercise in physically unfit but otherwise healthy men compared to fit healthy men (20). Elevated levels of creatine kinase (CK) were noted three days later. Since CFS patients are often inactive and physically deconditioned (18, 19), there may be an etiological link between physical deconditioning and activity-induced cytokine production in patients with the chronic fatigue syndrome (21, 22).

In the current investigation, we tested two pre-hoc hypotheses: (1) that a maximal exercise protocol would have a greater effect on certain cytokines than sub-maximal exercise in patients with CFS, (2) that certain cytokines are released more in CFS patients after exercise than in sedentary healthy controls. After noting sustained elevations of TGF-β before exercise testing, we also went on to test a further hypothesis; that CFS patients have an elevated response to TGF-β after the activity involved in traveling to the study center.

**MATERIALS AND METHODS**

**Subjects and Controls**

Nine patients with CFS, diagnosed according to Fukuda et al. criteria (2), were studied with the modification of having no co-morbid psychiatric disorder. All psychiatric disorders were excluded using the Standard Clinical Interview for DSM IV (SCID) (23) given by a psychiatrist. Eligible and willing subjects were chosen consecutively from two secondary care chronic fatigue clinics, situated in the departments of psychiatry and immunology at the same teaching hospital. Nine healthy but sedentary (moderate exercise for 20 minutes less than once a week) controls were chosen from hospital staff at the same hospital. Controls were matched by age, gender and exercise test (sub-maximal or maximal effort). All subjects had to travel from their homes to the center for testing. All subjects gave written informed consent. The study was approved by the East London and the City research ethics committee.
**Protocol**

Subjects were studied before and up to three days after a standardized bicycle ergometer exercise task (see Figure 1). The CFS and control subjects were assigned to either a maximal incremental exercise to exhaustion (“max” test) or a sub-maximal exercise test at 70% of age predicted maximum heart rate (HR) (“sub-max” test). Patients were ran-

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FIGURE 1. Flow diagram of study.

9 CFS patients

9 healthy controls

Baseline measures on day 1

Exercise testing on day 2

4 cases and controls had maximum testing; 5 cases and controls had sub-maximal testing

Blood taken on day 5 (3 days after exercise testing)

7 of the original CFS cases had blood taken, several months later, before and after traveling to hospital
domized to one or other protocol, and controls were matched to CFS cases by the exercise protocol. The two different exercise protocols were conducted to examine whether there were any different physiological responses to different intensities and duration of exercise testing. Because of the severity of fatigue in the CFS subjects, we were only able to undertake a single exercise test. All subjects had blood taken for laboratory measures and completed questionnaires on day one, day two (immediately before and after exercise testing, and 3 hours after the exercise test), and day five. Blood samples were taken between 9:30 a.m. and 12:30 p.m., except the three hour post-exercise sample on day two.

Some months later, we restudied seven of the original patients by taking venous blood at their homes first thing in the morning, before they had got out of bed. We then took a second sample on another day, after they had travelled across London to the study center (see Figure 1). Of the two missing patients, one had emigrated and the other was lost to follow-up.

Exercise Testing

All exercise testing was completed on a friction loaded cycle ergometer (Monark, 808, Sweden). All subjects completed a five minute familiarization period of unloaded cycling at a cadence of 60 rev min\(^{-1}\). After the familiarization period, the subjects completed either the maximal incremental or sub-maximal exercise protocol, chosen randomly. In the maximal incremental protocol (undertaken by four cases and controls), the required workload was initially 30 watts (W) and was increased by 30 W every two minutes until exhaustion. The subjects were encouraged to exercise until voluntary exhaustion. In the sub-maximal exercise protocol (undertaken by five cases and controls), the workload was increased by 30 W every two minutes until a HR equal to 70% of age predicted maximum HR (220–age in years) was achieved. After this point, the subjects exercised at this workload for 20 minutes or until exhaustion. Volitional exhaustion was considered to have occurred if the subject was no longer able maintain the required cadence, in spite of encouragement. The cycle ergometer was chosen in preference to a motorized treadmill because this device requires limited familiarization and has previously been used to determine maximal oxygen uptake (\(\text{VO}_2\) max) in CFS sufferers.

During each exercise test subjects breathed through a mouthpiece connected to a system of open circuit spirometry. Expired gases were
analyzed breath-by-breath throughout the exercise by a mass spectrometer (CPX/D, Medical Graphics Inc., USA) calibrated with known gases and volumes prior to each test. The oxygen consumption (VO$_2$) (ml kg min$^{-1}$) was averaged every 30 seconds with peak VO$_2$ determined as the highest VO$_2$ value obtained during any 60-second period of the test. Heart rate (HR) was also measured continuously throughout exercise via a modified three lead electrocardiogram then averaged every 30-s. The highest 30 second average HR (b min$^{-1}$) was deemed to be the maximum HR (HRmax). The exercise duration (seconds) for both the submaximal and maximal exercise task was also recorded.

**Laboratory Measures**

Three cytokines were measured on the basis that either they would be expected to be abnormally regulated in CFS after exertion and reflected a broad range of cell types, or that they had been previously shown to be elevated in CFS (6-9). Plasma concentrations of cytokines were analyzed by ELISA methodology according to manufacturer’s instructions, and their supporting data (R & D Systems). They included transforming growth factor-beta 1 (TGF-$\beta$1), tumor necrosis factor-alpha (TNF-$\alpha$), both found in many cell types, and mainly monocyte-derived interleukin-1 alpha (IL-$\alpha$). Plasma concentrations were measured in the exercise study, but serum levels were taken before and after traveling.

Cells within the immune system were measured by flow cytometry, as marker populations for cytokine dysregulation. These included lymphocyte sub-populations, using CD3, CD4, and CD8 cell markers, and HLA DR expression, as an activation marker.

Serum creatine kinase (CK) was measured by the normal method. Laboratory and exercise testing researchers were blind to group membership for the last 10 subjects tested, but not the first eight.

**Symptomatic Measures**

Fatigue was measured with the Chalder fatigue questionnaire at baseline and at final interview (24). Perceived physical disability was measured by the Medical Outcome Study Short Form (SF-36) physical function scale (25). These measures are well validated and reliable measures in CFS (3). The McGill pain score scale was used to measure
change in pain intensity with exercise (26), and an ad hoc five item Likert scale to measure the effect of exercise on delayed fatigue (available from authors).

**Statistical Analysis**

Since there were so few subjects, the data were not normally distributed. Therefore, continuous data were compared with the Mann-Whitney test with two tailed probability testing. Since this was a pilot study, results were considered statistically significant if p < 0.10. The response to exercise testing (max or sub-max) within groups was compared by the Wilcoxon matched pairs signed rank test, using the baseline of pre-exercise measures on day two. The inter-group measures were compared by calculating the area under the curve on the day of the exercise test, in order to avoid multiple comparisons. The percentage change (delta) from baseline readings on day two was compared, in order to examine change with exercise between groups. The Wilcoxon matched pairs signed rank test was used to compare data before and after traveling to hospital.

**RESULTS**

There were five women and four men in both groups. The median (inter-quartile) (IQR) age of the subjects was 36 (33-50) years and 38 (30-48) in the controls. Symptom scores of patients and controls are given in Table 1.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CFS</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chalder fatigue score</td>
<td>10 (8.5-10.5)</td>
<td>0 (0-0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>SF36 physical function</td>
<td>40 (35-53)</td>
<td>100 (92-100)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Pain at baseline</td>
<td>2 (1-2)</td>
<td>0 (0-0)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Pain 3 hours after exercise</td>
<td>2 (1.5-3)</td>
<td>1 (0-1.5)</td>
<td>0.008</td>
</tr>
<tr>
<td>Pain 3 days after exercise</td>
<td>2 (1-3.5)</td>
<td>0 (0-0)</td>
<td>0.0003</td>
</tr>
<tr>
<td>Fatigue 3 days after exercise</td>
<td>4 (4-4.75)</td>
<td>0 (0-0.5)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

Figures are medians (inter-quartile ranges). P value by Mann-Whitney test.
The Effects of the Exercise Test

Subjects exercising maximally spent a median (IQR) of 330 (300-420) secs on the bicycle, compared to 1200 (600-1230) secs in the sub-max group (p < 0.001). The median (IQR) final power was 210 (180-240) W in the maximal exercise group, compared to 60 (30-120) W in the sub-max group (p < 0.001). The median (IQR) HRmax was 178 (155-190) b min$^{-1}$ in the maximal exercise group versus 158 (141-166) b min$^{-1}$ in the sub-max group (p = 0.03). The median (IQR) VO$_2$ peak was 28.9 (20.6-30.0) ml kg min$^{-1}$ in the maximal exercise group versus 18.5 (12.7-20.7) ml kg min$^{-1}$ in the sub-max group (p = 0.02). There were no statistically or clinically significant differences in immune measures or muscle protein concentrations between the two exercise protocols.

The physiological data obtained from the exercise testing for the two groups are given in Table 2. There were no statistically significant differences between CFS patients and control subjects. Both patients and controls noted that the exercise was abnormal activity for them. There was no significant change in pain scores with exercise, but fatigue scores increased three days after exercise. Most patients anecdotally reported that the exercise test had a subsequent negative effect on their health and disability, of up to two weeks duration, indicating that the exercise level was of a type and intensity sufficient to trigger a symptomatic set-back.

There were no statistically or clinically significant differences in CK concentrations between groups or over time.

Figures 2 and 3 show the results for the absolute CD3, DR, and CD4, CD8 counts in both groups, showing significantly lower counts in CFS patients for CD3, CD4 and HLA DR even at baseline. The area under the curve was significantly lower in CFS patients compared to controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CFS</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time on bicycle (seconds)</td>
<td>510 (420-870)</td>
<td>810 (300-1200)</td>
<td>0.96</td>
</tr>
<tr>
<td>Maximum power achieved (watts)</td>
<td>150 (60-210)</td>
<td>120 (30-180)</td>
<td>0.96</td>
</tr>
<tr>
<td>Maximum heart rate (bpm)</td>
<td>161 (146-177)</td>
<td>173 (142-188)</td>
<td>0.45</td>
</tr>
<tr>
<td>Peak VO$_2$ (ml/kg/min)</td>
<td>22.9 (13.7-25.2)</td>
<td>20.6 (16.5-29.4)</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Median (inter-quartile range). Mann-Whitney test p values. Note: These data comprise both maximal and sub-maximal exercise data combined.
FIGURE 2. Median CD3 and DR counts before and after exercise testing.

FIGURE 3. Median CD4 and CD8 counts before and after exercise testing.
for CD3 (p = 0.02), CD4 (p = 0.02) and HLA DR (p = 0.005), but only a trend for CD8 (p = 0.12). The percentage changes as a response to exercise were not significantly different between groups for CD3 (p = 0.35), CD4 (p = 0.12), CD8 (p = 0.35) or HLA DR (p = 0.12). Within both groups exercise induced an immediate but not sustained significant increase in circulating counts for all four T cell markers. CD3 (p = 0.01), CD4 (p = 0.01), CD8 (p = 0.02), DR (p = 0.01) for CFS patients, with similar significant changes in controls (Figures 2 and 3).

Concentrations of TGF-β1 were significantly elevated in CFS patients at all times before and after exercise testing including the two baselines on days one and two (Figure 4). The area under the curve was significantly greater (p < 0.001). However, the percentage change in response to exercise was not significantly different (p = 0.92). Similarly, within the CFS patients there were no significant changes (p > 0.20) in TGF-β1 concentrations after exercise immediately after exercise, and three hours later. In addition there were no significant (p = 0.14) changes on day five (Figure 4).

The only significant differences in TNF-α between patients and controls were at 3 hours and 3 days after exercise (p = 0.06 and 0.03, respec-

**FIGURE 4.** Median TGF-β concentrations (pg/ml) before and after exercise testing.
tively) (Figure 5), but there were no significant differences between groups before these times. These differences resulted in the area under the curve being significantly different between groups ($p = 0.004$). Within the CFS group alone, there were significantly higher concentrations at 3 hours ($p = 0.04$), which were sustained 3 days after exercise ($p = 0.02$) (Figure 5). There were no significant differences in interleukin $1\alpha$ between groups, or any within groups over time (data not shown).

The Effects of Traveling to the Hospital

The median (IQR) Chalder fatigue scale score of the seven patients, at the time of further testing, was 9 out of a maximum of 11 (7-11), which was similar to the previous median score of 10. The median (IQR) SF-36 physical function sub-scale score was 35 (20-55), which was similar to the previous score of 40.

Table 3 gives the data for TGF-β before getting up in the morning and after traveling to the hospital. This shows a statistically significant median (IQR) elevation in TGF-β after traveling of 435 pg/ml (26-1720)

FIGURE 5. Median TNF-α concentrations (pg/ml) before and after exercise testing.
We found no significant associations between TGF-β concentrations and any of the clinical measures. There were no significant differences between the median TNF-α, IL-1α, nor CPK concentrations before compared to after travel (data not shown).

**DISCUSSION**

The main finding of this pilot study was the elevated median concentration of transforming growth factor beta, which seemed to be related to activity. We also found significantly fewer CD3+ and CD4+ lymphocytes and fewer expressing HLA DR, but there was no difference between groups in response to exercise, but no importance should be attached to this in view of the small numbers of subjects in this study. Finally, we found that exercise induced a sustained elevation in the concentration of TNF-α, which was still present three days later, and this only occurred in CFS patients.

TGF-β was grossly elevated when compared to controls before exercise, and showed no differential response to exercise, but did show an increase in response to the exercise entailed in getting to the study center. These data replicate three out of four previous studies finding elevated TGF-β in subjects with CFS (6, 7, 9, 10). Our data are consistent with that of Peterson and colleagues, who found the effect of exercise on serum TGF-β appeared to be magnified in CFS patients (9). Inoue and colleagues used a murine model to show that exercise induces CSF TGF-β, which in turn causes motor inactivity when injected intracerebroventricularly into other mice (12). The elevating effect on TGF-β of traveling to hospital may explain the baseline pre-exercise testing elevation of TGF-β, with such activity being unusual for our patients.

Although we did not replicate the previous finding of elevated baseline concentrations of TNF-α in subjects with CFS, we did find that exercise induced significantly elevated concentrations; a finding also noted in healthy but unfit men undertaking exercise (17). The pro-inflammatory TNF-α is known to be a cause of acute sickness behavior, characterized by reduced activity related to “weakness, malaise, listlessness and inability to concentrate” (27), symptoms also notable in CFS (1, 2).

On a technical note, we found no differences in immune or muscle responses between a longer 70% sub-maximal exercise test and a shorter 100% maximal test of exercise endurance. Both of these exercise tests were sufficiently abnormal stressors, compared to usual activity in our...
patients, and suggests that future studies might use either method with the advantage of the maximal test being that maximal aerobic capacity can be established. Patients were very fatigued with both this and reported physical disability consistent with other studies of CFS (16, 18, 19).

These preliminary findings require replication in a larger single blind case-control study before we can judge their significance, particularly since we were aware of case-control status for some subjects. These preliminary data suggest that “ordinary” activity (i.e., that involved in getting up and traveling some distance) may induce anti-inflammatory cytokine release (TGF-β), whereas more intense exercise may induce pro-inflammatory cytokine release (TNF-α) in patients with CFS (21, 28, 29). The causal mechanisms involved and the direction of the relationship between these mechanisms remain to be elucidated. Altered cytokine balance, for example, following an infection, may modify the threshold at which cytokine release occurs with exercise or activity, setting up a vicious circle. These processes could contribute to the post-exertional malaise, myalgia and the central fatigue that characterize CFS (1, 2, 4). Future studies should study patients at truly resting baseline levels, over a longer time-course, and should examine gene expression of cytokines, as well as circulating levels (17).

REFERENCES


**TABLE 3. TGF-β (pg/ml) before and after traveling to hospital.**

<table>
<thead>
<tr>
<th>Patient code</th>
<th>Before</th>
<th>After</th>
<th>Difference</th>
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<tbody>
<tr>
<td>B</td>
<td>1161</td>
<td>1596</td>
<td>+ 435</td>
</tr>
<tr>
<td>C</td>
<td>129</td>
<td>1070</td>
<td>+ 941</td>
</tr>
<tr>
<td>D</td>
<td>1246</td>
<td>1328</td>
<td>+ 82</td>
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<tr>
<td>E</td>
<td>184</td>
<td>2206</td>
<td>+ 2022</td>
</tr>
<tr>
<td>F</td>
<td>1129</td>
<td>1155</td>
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<tr>
<td>G</td>
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<td>1363</td>
<td>+ 8</td>
</tr>
<tr>
<td>H</td>
<td>48</td>
<td>1768</td>
<td>+ 1720</td>
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<tr>
<td>Median</td>
<td>1129</td>
<td>1363</td>
<td>+ 435</td>
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